Environmental Pollution 244 (2019) 367–378

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Two decades of monitoring in marine debris ingestion in loggerhead sea turtle, *Caretta caretta*, from the western Mediterranean[☆]

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ARTICLE INFO

Article history:
Received 10 June 2018
Received in revised form
8 October 2018
Accepted 9 October 2018
Available online 15 October 2018

Keywords:
Marine debris ingestion
Caretta caretta
Good environmental status
Plastic
Mediterranean sea

ABSTRACT

Anthropogenic marine debris is one of the major worldwide threats to marine ecosystems. The EU Marine Strategy Framework Directive (MSFD) has established a protocol for data collection on marine debris from the gut contents of the loggerhead sea turtle (*Caretta caretta*), and for determining assessment values of plastics for Good Environmental Status (GES). GES values are calculated as percent turtles having more than average plastic weight per turtle. In the present study, we quantify marine debris ingestion in 155 loggerhead sea turtles collected in the period 1995–2016 in waters of western Mediterranean (North-east Spain). The study aims (1) to update and standardize debris ingestion data available from this area, (2) to analyse this issue over two decades using Zero-altered (hurdle) models and (3) to provide new data to compare the only GES value available (off Italian waters). The composition of marine debris (occurrence and amounts of different categories) was similar to that found in other studies for the western Mediterranean and their amounts seem not to be an important threat to turtle survival in the region. Model results suggest that, in the study area, (a) period of stranding or capture, (b) turtle size and (c) latitude are significant predictors of anthropogenic debris ingestion (occurrence and amount) in turtles. The GES value for late juvenile turtles ($CCL>40$ cm) has decreased in the last ten years in the study area, and this is very similar to that obtained in Italian waters. We also provide a GES value for early juvenile turtles ($CCL\leq40$ cm) for the first time. Recommendations arising from this study include ensuring use of (1) the standardized protocol proposed by the MSFD for assessing marine debris ingestion by loggerhead sea turtles and (2) the ecology of the turtles (neritic vs oceanic), rather than their size, to obtain GES values.

1. Introduction

Any waste of natural or human origin found in marine or coastal environments is considered marine debris (Laist, 1997; MSFD et al., 2011). Anthropogenic marine debris is recognized as one of the greatest threats to marine ecosystems that compromise biodiversity and marine resources (Moore, 2008; Galgani et al., 2010; Sutherland et al., 2010; UNEP, 2011; CBD, 2012). Several million tons of anthropogenic debris reach the oceans annually, leaving critical amounts of items floating at the surface, deposited on the sea bottom at all depths, and stranded on coasts throughout the world (e.g., Ryan, 2013; Schlining et al., 2013; Suaria and Aliani, 2014).

Ingestion of anthropogenic marine debris by marine vertebrates is an increasingly recognized phenomenon (Laist, 1997; Derraik, 2002; Gall and Thompson, 2015; Kühn et al., 2015). Marine turtles, in particular, appear to consume anthropogenic debris because they confuse it with food (Campani et al., 2013; Schuyler et al., 2015) and, not surprisingly, all species have been reported to have debris in their stomach contents (Gall and Thompson, 2015; Schuyler et al., 2015). Marine debris ingestion can cause sub-lethal or even lethal effects in these animals. Sub-lethal effects include dietary dilution or assimilation of contaminants derived from marine litter (Bjorndal, 1997; McCauley and Bjorndal, 1999). In severe cases, debris can block or tear their digestive tracts resulting in the death of the turtles (Bjorndal et al., 1994; Tomás et al., 2002; Lazar and Gračan, 2011; Vélez-Rubio et al., 2018).

The Mediterranean Sea represents a semi-closed basin with high demographic density throughout its entire coastline and is one of the most polluted seas of the planet (Suaria and Aliani, 2014; Pham

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☆ This paper has been recommended for acceptance by Maria Cristina Fossi.

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https://doi.org/10.1016/j.envpol.2018.10.047
0269-7491/

et al., 2014; Cózar et al., 2015). The European Commission recently adopted a Marine Strategy Framework Directive (MSFD) (European Directive, 2008) aimed at achieving a Good Environmental Status (GES) in 2020 for the Mediterranean and the rest of European seas and oceans (Galgani et al., 2014; Matiddi et al., 2017). One of the 11 qualitative descriptors in the MSFD included the study of trends in the amount and composition of marine debris ingested by marine animals. To do this, the MSFD adopted the methodology used by Van Franeker (2004) and Van Franeker et al. (2011), which has been used to monitor the long-term ingestion of anthropogenic debris by the North fulmar, *Fulmarus glacialis*. The MSFD also proposed the use of the loggerhead sea turtle, *Caretta caretta*, as an indicator species of pollution in the Mediterranean Sea (MSFD et al., 2013). The loggerhead sea turtle was considered as an eligible indicator species for four reasons: (1) it is the most abundant sea turtle species in the Mediterranean (Casale and Margaritoulis, 2010), (2) it has been used as an indicator of pollution in other regions of the world (Aguirre and Lutz, 2004; Keller et al., 2006; Foti et al., 2009), (3) a large number of accidental catches of this species occur in this basin (Casale, 2011), and (4) there is data available on many individual turtle from recovery centers and stranding networks (Ullmann and Stachowitsch, 2015).

Six studies have hitherto analysed the ingestion of marine debris in the loggerhead sea turtle adopting the MSFD methodology: (1) in the North-east Atlantic Ocean region, one study was carried out in the Bay of Biscay and Atlantic Iberian Coast subregion (Nicolau et al., 2016) and one in the Macaronesia subregion (Pham et al., 2017); (2) in the Mediterranean Sea region, one study was developed in the Ionian Sea and Central Mediterranean sub-region (Italy; Casale et al., 2016) and three in the Western Mediterranean sub-region (Italy; Campani et al., 2013; Camedda et al., 2014; Matiddi et al., 2017). These studies report debris ingestion at specific times, but provide no data over long periods (i.e., decades). However, long-term series are necessary to obtain robust evidence on population trends and changes in the habitat use of species (Durant et al., 2007; Aznar et al., 2017). In particular, long-term data provides fundamental insight to understand potential changes in the ingestion of marine debris and its impact on marine vertebrates such as the loggerhead sea turtle.

The MSFD has proposed to establish a Good Environmental Status (GES) using the loggerhead sea turtle, based on Ecological Quality Objectives for the North Sea (EcoQO's) obtained from the abundance of plastics in stomachs of seabirds (Van Franeker et al., 2011). For the first time, Matiddi et al. (2017) applied a specific methodology to obtain a value of GES, i.e., the percent turtles having more than average plastic weight per turtle, using samples of 50–100 turtles. These authors proposed to distinguish separated GES scenarios for early juvenile turtles ($CCL \leq 40$ cm) and for subadults and adult ones ($CCL > 40$ cm) according to generic ontogenetic differences in sea turtle feeding ecology (Bjorndal, 1997; Frick et al., 2009; Lazar et al., 2011).

Here we analyse, for the first time, data on anthropogenic debris consumption by the loggerhead sea turtle collected over two decades in the Western Mediterranean sub-region, applying the MSFD methodology. We also provide and discuss the GES values obtained so far to assess trends on debris ingestion by loggerhead turtles in the western Mediterranean.

2. Materials and methods

2.1. Sample collection

Gut contents of 155 loggerhead sea turtles (*Caretta caretta*) were examined for marine debris ingestion. Individuals were collected dead by the cetacean and sea turtle stranding network of the Valencia region, East Spain, between 1995 and 2016. Turtles were found stranded on the coast ($n = 68$) or accidentally caught by pelagic long-line, bottom trawl or artisanal fisheries (mainly trammel nets) ($n = 87$) operating in waters of this region. Standardized morphometric measurements and subsequent necropsy of each turtle were made following Wyneken and Witherington (2001) protocol. During the necropsies, the entire digestive tract was removed, and carefully dissected and washed over a 0.5 mm light sieve. Solid contents were preserved in 70% ethanol for later identification and quantification.

Solid contents were analysed under a stereo-microscope and divided into 5 categories, following the protocol described in the MSFD et al. (2013) for marine turtles: (1) Plastics, (2) Rubbish other than plastics, (3) Pollutants, (4) Natural food remains, (5) Natural non-food remains. A description of each category and its subcategories used in the present study followed the '*Appendix Supplemental file*' provided by Van Franeker et al. (2011). Dietary items (category 4) were preserved in 70% ethanol for future studies on trophic ecology. The remaining four categories were collectively considered as 'debris' (meso- and macro-debris *sensu stricto*, i.e. particles ≥ 1 mm long; see Ryan, 2016): Items from categories from 1 to 3 were considered as 'anthropogenic debris' and those from category 5 as 'natural debris' (e.g., rocks, feathers and other natural items that cannot be considered as food).

For each subcategory of debris, we obtained four parameters per turtle; number of items, wet volume, dry weight, and body burden. Wet volume (to the nearest 0.1 ml) was calculated by immersing debris items in a beaker with a known volume of distilled water. Dry weight was measured by drying debris items at 24 °C for 48 h, then weighing them with a Shimadzu UX 420H precision scale (to the nearest 0.001 g). Body burden was calculated as g of marine debris divided by kg of turtle (Clukey et al., 2017; White et al., 2018). Of the 155 turtles analysed, the weight of 81 turtles was available. We calculated a linear regression of Curved Carapace Length [CCL] (cm) over weight (kg) using ln-transformed variables to estimate the weight of the remaining 74 turtles. The result equation was $\text{ln-weight} = 2.903\ \text{lnCCL} - 8.690$ ($R^2 = 0.982$, $n = 81$, $p < 0.00001$). A correction factor was applied after the back transformation of raw values following Sprugel (1983). Mean (±SD), median and range for number of items, wet volume, dry weight and body burden were calculated for each debris category, and the 95% Confidence interval (CI) of frequency of occurrence was estimated with Sterne's exact method (Reiczigel, 2003) using the free software Quantitative Parasitology v.3 (Reiczigel and Rózsa, 2005).

2.2. Predictors of debris ingestion

Dry weight was measured with more accuracy than number of items or wet volume and, therefore, dry weight (g) of anthropogenic debris (i.e., Plastics, Rubbish and Pollutants) per turtle was used as response variable in statistical analyses. The following predictors were considered (see Table 1 for details): (1) Period of stranding or capture. Here, years were pooled into two groups; 1995–2004 and 2005–2016 because the sample size was small for several years and results could be interpreted more easily for the purposes of the study (see the Discussion); (2) Season; (3) Curved Carapace Length (notch to tip; CCL); (4) Latitude, and (5) Origin (stranded or bycaught). The type of fishing gear (pelagic longline, bottom trawling or artisanal fisheries) was not included as a predictor because it was confounded with 'period' (Fig. 1).

The distribution of anthropogenic debris mass in the digestive contents of the turtles sampled was right-skewed, with 26.2% of zero values (Fig. 2). Thus, we used hurdle models (or Zero-altered models) for continuous data, i.e., Zero-altered gamma (ZAG) and Zero-altered lognormal (ZALN) (Taranu et al., 2017; Zuur and Ieno,

Table 1
Description and identification of response and explanatory variables (covariates) used in Zero-altered Gamma (ZAG) and Zero-altered Lognormal (ZALN) models to model the weight of anthropogenic debris ingested by loggerhead sea turtles, *Caretta caretta* in the Valencia region (East Spain).

Variable	Description	Type
Response		
DEBRIS	Weight (g) of anthropogenic debris in each digestive content.	Continuous
Explanatory		
PERIOD	Period of years of turtle death.	Categorical (*2 levels*) *1.1995-2004* *2.2005-2016*
CCL	Curved carapace length (cm) of the turtle.	Continuous
LATITUDE	Latitude	Continuous
SEASON	Turtle death season (according to the Northern Hemisphere).	Categorical (*4 levels*) *1. Dec. 21 – Mar. 20* *2. Mar. 21 – June 20* *3. June 21 – Sept. 22* *4. Sept. 23 – Dec. 20*
ORIGIN	Cause of turtle entry into the stranding network.	Categorical (*2 levels*) *1. Stranded* *2. Bycaught*

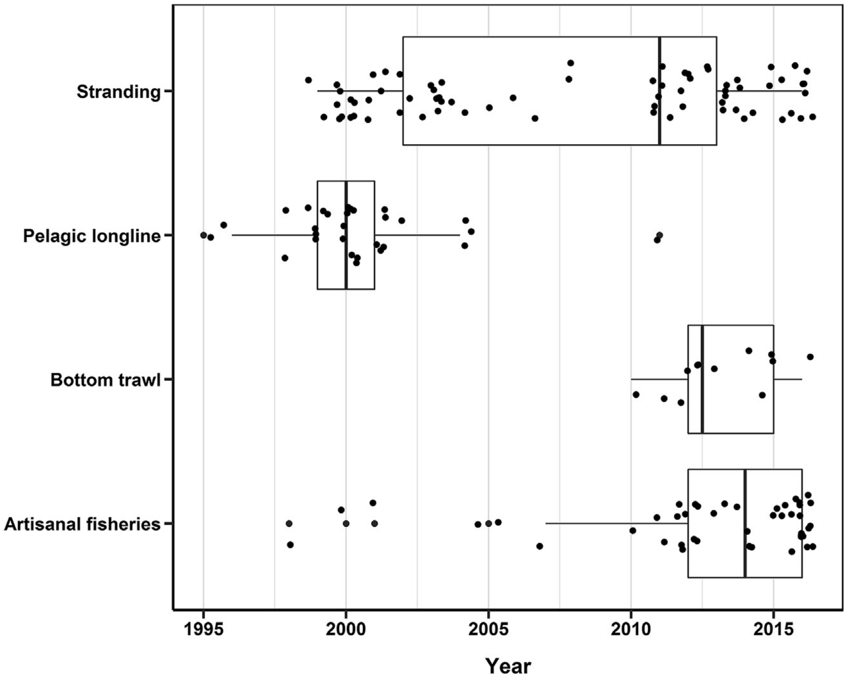

Fig. 1. Cause of entry of 155 loggerhead sea turtles, *Caretta caretta*, recorded dead by the stranding network of the Valencia region (East Spain) between 1995 and 2016.

2016). In particular, a hurdle model consists in two parts to conjointly deal with the zero and non-zero values of debris mass. The first part consists in a Bernoulli GLM to model the process that caused the absence and presence of anthropogenic debris. The second part, uses a GLM with Gamma or Lognormal distribution to model data for zero-truncated dry weight of anthropogenic debris. For each part of hurdle models, all predictors included in Table 1 were tested.

Models were compared using values of Akaike Information Criterion (AIC). The AIC value of each model was obtained through the sum of AIC values of the Bernoulli and the Lognormal or Gamma parts of each ZALN and ZAG model (Zuur and Ieno, 2016). The model with minimum AIC was considered the “best model”, and the rest of the models were ranked according to differences in their AIC values (Johnson and Omland, 2004). Models with values of $\Delta\text{AIC} \leq 2$ were considered to have substantial empirical support, whereas those having $\Delta\text{AIC} > 2$ were assumed to have much less support (Burnham and Anderson, 2002). It was also assumed that models with Akaike weights $(w_i) \leq 0.100$ were unlikely to be the “true” models (Burnham and Anderson, 2002).

The best-adjusted model was validated by assessing the patterns in the fitted values, predicted values, and residuals, based on the expected values of the combined model, i.e., considering the Bernoulli part and the Lognormal or Gamma part conjointly (Zuur and Ieno, 2016). For model diagnostics and subsequent predictions, mean and variance of the best-adjusted model was calculated by combining the mean and variance of the Bernoulli and the Lognormal or Gamma parts following Zuur and Ieno (2016).

Analyses and graphics were carried out using free software R (R Core Team, 2016) through open source program RStudio (RStudio Team, 2016). The Bernoulli, Lognormal or Gamma parts of Hurdle models were calculated separately and then combined using the

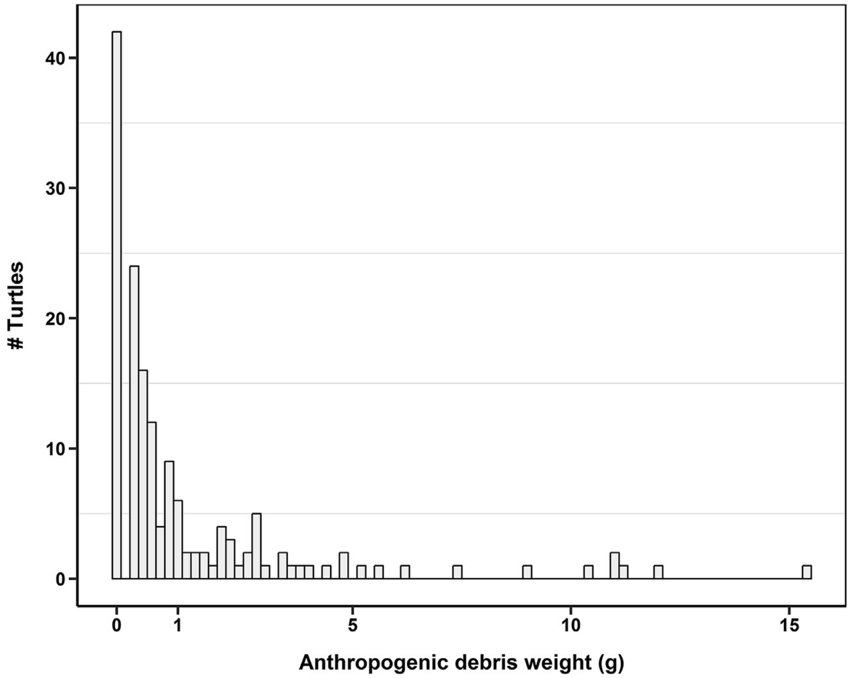

Fig. 2. Frequencies of anthropogenic debris weight in the digestive contents of 155 loggerhead sea turtles, *Caretta caretta* from Valencia region (East Spain).

‘stats’ package (R Core Team, 2016). Graphical model visualizations were performed by means of ‘ggplot2’ package (Wickham, 2009).

2.3. Assessment of Good Environmental Status

Following Matiddi et al. (2017), we obtained GES values as percent turtles having more than average plastic weight per turtle, using samples of 50–100 turtles. In order to make the results comparable with Matiddi et al. (2017), in the present study we used the same methodology to calculate GES values, although this methodology will be discussed latter. We decided to calculate GES values every 10 years since samples were available for the period 1995–2016. Accordingly, ‘*Past GES*’ (1995–2005) and ‘*Current GES*’ (2006–2016) were both obtained for early juveniles (CCL ≤40 cm) and late juveniles (CCL >40 cm).

3. Results

3.1. Turtle size distribution

The 155 turtles analysed in the present study were mainly juveniles (mean Curved Carapace Length [CCL] ± SD [Range] = 51.1 ± 14.9 [11.0–80.0] cm) assuming that 70 cm is the minimum CCL for adult loggerhead turtles from Mediterranean or Atlantic populations inhabiting Mediterranean Sea (Margaritoulis et al., 2003; Casale et al., 2011). For the period 1995–2005 (mean CCL ± SD [Range] = 57.4 ± 11.4 [32.0–79.0] cm, N = 60), turtles were significantly larger (Mann-Whitney test, U = 4289, N = 155, $p < 0.001$) than the turtles from the 2006–2016 period (mean CCL ± SD [Range] = 47.0 ± 15.1 [11.0–80.0] cm, N = 95). Oceanic turtles captured by pelagic longline (mean CCL ± SD [Range] = 60.1 ± 11.7 [37.0–79.0] cm, N = 29) were significantly larger (Mann-Whitney test, U = 1650.5, N = 155, $p < 0.001$) than neritic turtles captured by bottom trawling and trammel nets (mean CCL ± SD [Range] = 46.3 ± 13.6 [26.8–80.0] cm, N = 56).

3.2. Debris description

A total of 4423 debris items of 10 out the 15 debris subcategories were collected in 121 (78.1%) of the 155 turtles analysed, with a total dry weight of 406.9 g and a wet volume of 930.3 ml (Table 2). Plastics and natural non-food remains were the most frequent debris categories occurring in 69.0% and 60.6%, respectively (Table 2). All plastic remains (1414 items) were user plastics, with no traces of industrial plastic pellets, and accounted for 172.6 g (42.4% of total dry weight) and 416.2 ml (44.7% of total wet volume). Rubbish accounted for 31.3 g (7.7%) and 21.0 ml (2.3%), whereas the amount of pollutants (100% tar-lumps) were almost negligible (0.891 g and 2.7 ml); only one turtle presented the digestive tract partially covered by 9 ml of petroleum. Finally, natural non-food remains accounted for 202.2 g (49.7%) and 481.4 ml (51.7%). One turtle presented substantially more debris than usual in its gut contents (>15 g); debris items in this turtle appeared amalgamated in the intestine and could be causing intestinal blockage (CCL = 51.5 cm).

3.3. Predictors of debris ingestion

Candidate ZAG models outperformed equivalent candidate ZALN models for all models of anthropogenic debris ingestion tested (Table S1). Diagnostic plots of the best-adjusted ZAG model did not show strong patterns in (a) Pearson residuals vs. expected ZAG values, (b) response variable vs. expected ZAG values and, (c) Pearson residuals vs. covariates (Fig. S1). Best-fitted ZAG models were ranked according to AIC (Table 3). Five candidate models had substantial empirical support ($\Delta AIC \leq 2$), and included four predictors, i.e., 'period', ‘CCL’, 'latitude', and 'origin' (Table 3). The best model included the four predictors in the Gamma part, and only ‘period’ in the Bernouilli part (Table 3); all predictors except ‘origin’ were statistically significant ($p < 0.01$) (Table 4). All the remaining 4 models always included ‘period’ in the Bernouilli part and ‘period’, ‘CCL’ and ‘latitude’ in the Gamma part (Table 3); again, these predictors were all significant ($p < 0.05$) (Table 4).

Table 2
Debris ingestion descriptors in loggerhead sea turtles, *Caretta caretta*, from the Valencia region, East Spain, western Mediterranean (N = 155). Debris categories are based on Van Franeker et al. (2011). No traces of the categories industrial plastic (IND), human food (kit) wastes or coal or slag (coal) were found in the digestive contents of the turtles analysed; hence these are not included in the table. Only one turtle was found with non-natural chemicals (che) and due to its consistency only its volume was measured; therefore, the 'che' subcategory is also not included in the table. The methodology used to measure dry weight (in grams) and wet volume (in milliliters) can be seen in the materials and methods section. Mean (±SD) and median were calculated including turtles without debris; whereas for the range only turtles with debris in each category were considered. Acronyms: Mean (*M*), Median (*Mdn*), Range (*Rng*).

Debris categories	Frequency (%) (95% CI)	Dry weight (g)			Wet volume (ml)			Number of items			Body burden (g/kg turtle)		
		M (±SD)	Mdn	Rng	M (±SD)	Mdn	Rng	M (±SD)	Mdn	Rng	M (±SD)	Mdn	Rng
Sheetlike user plastics	60.6% (52.6–68.1)	0.353 (±0.922)	0.021	0.001–9.229	1.2 (±3.3)	0.1	0.1–30.0	4.5 (±6.7)	1	1–36	0.023 (±0.050)	0.002	0.001–0.268
Threadlike user plastics	31.6% (24.6–39.3)	0.110 (±0.443)	0.000	0.001–3.376	0.3 (±1.3)	0.0	0.1–13.0	0.9 (±2.5)	0	1–19	0.006 (±0.028)	0.000	0.001–0.253
Foamed user plastics	19.4% (13.8–26.4)	0.102 (±0.412)	0.000	0.001–3.035	0.3 (±1.6)	0.0	0.1–19.0	0.5 (±1.9)	0	1–11	0.007 (±0.031)	0.000	0.001–0.253
Fragments	38.7% (31.2–46.8)	0.393 (±1.159)	0.000	0.001–9.117	0.6 (±1.6)	0.0	0.1–11.0	2.7 (±5.9)	0	1–29	0.038 (±0.110)	0.000	0.001–0.809
Other	15.5% (10.6–22.2)	0.156 (±0.535)	0.000	0.036–3.288	0.3 (±1.1)	0.0	0.1–11.5	0.5 (±2.1)	0	1–23	0.011 (±0.042)	0.000	0.003–0.334
User plastic	69.0% (61.3–75.9)	1.113 (±2.312)	0.128	0.001–15.175	2.7 (±5.7)	0.5	0.1–38.0	9.1 (±12.8)	4	1–63	0.086 (±0.191)	0.010	0.001–1.265
Paper	2.6% (0.4–6.4)	0.003 (±0.020)	0.000	0.045–0.228	0.03 (±0.24)	0.0	0.2–2.6	0.03 (±0.16)	0	1	0.001 (±0.003)	0.000	0.003–0.029
Various rubbish	1.3% (0.2–4.7)	0.005 (±0.044)	0.000	0.286–0.462	0.005 (±0.056)	0.0	0.4–0.7	0.09 (±1.05)	0	1–13	0.001 (±0.002)	0.000	0.019–0.024
Fish hook	5.8% (2.6–10.9)	0.194 (±1.189)	0.000	0.076–10.599	0.09 (±0.67)	0.0	0.1–7.5	1 (±7.9)	0	1–95	0.007 (±0.039)	0.000	0.005–0.353
Rubbish	9.7% (5.7–10.9)	0.202 (±1.189)	0.000	0.045–10.599	0.1 (±0.7)	0.0	0.1–7.50	1.1 (±8.0)	0	1–95	0.008 (±0.039)	0.000	0.003–0.353
Pollutants *Tar-lumps*	5.8% (2.9–10.4)	0.006 (±0.033)	0.000	0.001–0.3070	0.02 (±0.11)	0.0	0.1–1.20	0.4 (±2.5)	0	1–25	0.001 (±0.002)	0.000	0.001–0.014
ANTHROPOGENIC DEBRIS	**71.0% (63.2–77.8)**	**1.321 (±2.629)**	**0.193**	**0.001–15.463**	**2.9 (±6.0)**	**0.5**	**0.1–38.2**	**10.6 (±16.3)**	**4**	**1–112**	**0.094 (±0.195)**	**0.019**	**0.001–1.288**
Natural non-food remains	60.6% (52.6–68.1)	1.305 (±3.356)	**0.029**	0.001–26.025	3.1 (±7.9)	**0.1**	0.1–66.4	17.9 (±38.5)	3	1–279	0.113 (±0.277)	0.003	0.001–1.778
TOTAL DEBRIS	**78.1% (70.7–83.9)**	**2.625 (±5.021)**	**0.715**	**0.005–34.359**	**6.0 (±12.0)**	**1.5**	**0.1–90.8**	**25.5 (±44.5)**	**12**	**1–279**	0.207 (±0.432)	**0.044**	**0.001–2.863**

Table 3
Best fitted Zero-altered Gamma models (ZAG) accounting for the effect of covariates (described in Table 1) on anthropogenic debris weight in digestive contents of loggerhead sea turtles, *Caretta caretta*, from the Valencia region (East Spain). Models with substantial empirical support ($\Delta AIC \le 2$) are highlighted in bold; those with much less support ($\Delta AIC > 2$) are on italic. Candidate models with $w_i \le 0.100$ are not shown.

Zero-altered gamma model (ZAG)			
Bernoulli part	*Gamma part*	ΔAIC	w_i
PERIOD	**PERIOD + CCL + LATITUDE + ORIGIN**	**0.00**	**0.786**
PERIOD	**PERIOD + CCL + LATITUDE**	**0.27**	**0.687**
PERIOD + LATITUDE	**PERIOD + CCL + LATITUDE + ORIGIN**	**1.39**	**0.393**
PERIOD + LATITUDE	**PERIOD + CCL + LATITUDE**	**1.66**	**0.343**
PERIOD + ORIGIN	**PERIOD + CCL + LATITUDE + ORIGIN**	**1.86**	**0.310**
PERIOD + LATITUDE + ORIGIN	*PERIOD + CCL + LATITUDE + ORIGIN*	*3.07*	*0.170*
PERIOD + LATITUDE + ORIGIN	*PERIOD + CCL + LATITUDE*	*3.34*	*0.148*
PERIOD + CCL + LATITUDE	*PERIOD + CCL + LATITUDE + ORIGIN*	*3.35*	*0.147*
PERIOD + CCL + ORIGIN	*PERIOD + CCL + LATITUDE + ORIGIN*	*3.79*	*0.119*

'Period' had a positive influence on the occurrence of anthropogenic debris (Table 4) increasing from 60% in 1995–2004 to 82% in 2005–2016 (Fig. 3a). In contrast, the mass of anthropogenic debris was significantly lower in 2005–2016 (Table 4, Fig. 3b). Turtle size (CCL) and 'latitude' positively affected the debris mass ingested, with larger turtles at the north of the study area containing significantly more debris (Table 4; Fig. 3c and d). As noted above, the effect of 'origin' (bycaught vs. stranded) was not unequivocal (Tables 3 and 4); with bycaught turtles having somewhat less total mass of anthropogenic debris (Fig. 3e). Expected values from the ZAG model, i.e., combining the Bernouilli and Gamma parts, indicated a similar, but less strong influence of the above predictors on the mass (including zeros) of anthropogenic debris (Fig. 3).

Zero-altered models considering only plastic remains rendered similar results (data not shown). The raw data from the analysed turtles can be consulted in Table S2.

3.4. Assessment of Good Environmental Status

Plastic ingestion parameters for early (n = 41) and late (n = 114) juveniles are shown in Table 5. The number of early juvenile individuals was slightly lower than the one proposed by Matiddi et al. (2017) to calculate the GES (n ≥ 50), and very few individuals of this

Table 4
Estimates and standard errors (SE) for Zero-altered Gamma models (ZAG) with substantial empirical support ($\Delta AIC \leq 2$). Those models account for the effect of covariates (described in Table 1) on anthropogenic debris mass in digestive contents of loggerhead sea turtles, *Caretta caretta*, from the Valencia region (East Spain). In bold values with significance ($p \leq 0.05$). Significance codes: †$p \leq 0.001$; ‡$p < 0.001–0.01$; *$p < 0.01–0.05$.

ΔAIC	Zero-altered gamma model (ZAG)					
	Bernoulli part			*Gamma part*		
	Variable	Estimator	SE	Variable	Estimator	SE
0.00	(Intercept)	0.405*	0.264	(Intercept)	**−31.732**†	7.856
	PERIOD *2005-2016*	**1.048**‡	0.371	PERIOD *2005-2016*	**−1.477**†	0.314
				CCL	**0.043**†	0.010
				LATITUDE	**0.788**†	0.198
				ORIGIN *Bycaught*	−0.414‡	0.282
0.27	(Intercept)	0.405*	0.264	(Intercept)	**−27.030**†	7.330
	PERIOD *2005-2016*	**1.048**‡	0.371	PERIOD *2005-2016*	**−1.403**†	0.310
				CCL	**0.042**†	0.010
				LATITUDE	**0.664**†	0.183
1.39	(Intercept)	−7.276	9.802	(Intercept)	**−31.732**†	7.856
	PERIOD *2005-2016*	**0.932***	0.398	PERIOD *2005-2016*	**−1.477**†	0.314
	LATITUDE	0.197*	0.251	CCL	**0.043**†	0.010
				LATITUDE	**0.788**†	0.198
				ORIGIN *Bycaught*	−0.414‡	0.282
1.66	(Intercept)	−7.276	9.802	(Intercept)	**−27.030**†	7.330
	PERIOD *2005-2016*	**0.932***	0.398	PERIOD *2005-2016*	**−1.403**†	0.310
	LATITUDE	0.197*	0.251	CCL	**0.042**†	0.010
				LATITUDE	**0.664**†	0.183
1.86	(Intercept)	0.478·	0.329	(Intercept)	**−31.732**†	7.856
	PERIOD *2005-2016*	**1.059**‡	0.373	PERIOD *2005-2016*	**−1.477**†	0.314
	ORIGIN *Bycaught*	−0.140·	0.376	CCL	**0.043**†	0.010
				LATITUDE	**0.788**†	0.198
				ORIGIN *Bycaught*	−0.414‡	0.282

category ($n = 3$) were available for the period 1995–2005. However, we elaborated the GES scenario for early juveniles from 2006 to 2016 because no data on this population segment are available for the Mediterranean. The GES value for early juveniles ($CCL \leq 40$ cm) for the period 2006–2016 ($n = 38$) indicated that 26.3% of turtles had more plastics (dry weight) than average (0.35 g).

The GES value for late juvenile turtles 1995–2005 ($n = 60$) indicated that 30.0% of turtles had more plastics (dry weight) than average (1.47 g). The percentage is similar for the period 2006–2016 ($n = 54$): 27.8% of turtles had more plastics than average (1.31 g). Accordingly, GES values suggest that, in the second period, there was a slight decrease in both the average amount of plastics ingested and the percent of late juvenile turtles having more plastic mass than average.

4. Discussion

4.1. Debris comparison

Ingestion of marine debris by loggerhead sea turtle has been reported in many regions of the world (see Schuyler et al., 2014; Nelms et al., 2016). The frequency of occurrence (FO%) of marine debris is generally below 50% (median = 35.2%, $n = 31$ studies; Table S3), but varies greatly depending on the region (Table S3), e.g. 0% in North-west Atlantic Ocean and North-east Pacific Ocean (Frick et al., 2001; Peckham et al., 2011), and 80% in central North Pacific Ocean (Clukey et al., 2017). Only some studies ($n = 14$) have quantified the amount of marine debris ingested by loggerhead sea turtles, and values are generally low (Table S3).

Variability in FO% is also high in the Mediterranean Sea, with values ranging from 2.4% to 85% in the eastern and western basins, respectively (Kaska et al., 2004; Matiddi et al., 2017, Table S3). The FO% of marine debris obtained in the present study (78.1%) is comparable to those obtained in other localities from the Spanish Mediterranean, i.e., 79.6% (Tomás et al., 2002) in northeast Spain, and 63.2% around Balearic Islands (Revellés et al., 2007). Mean dry weight of marine debris obtained in the present study (1.32 g) is similar to those obtained in other localities of western Mediterranean (Table S3).

Despite the high occurrence of debris in loggerhead sea turtles observed in the present study, we found little evidence that debris have caused impactions, obstructions or perforations in the gut. The small amount of debris is also suggestive of little dietary dilution (see Clukey et al., 2017). Lethal cases of ingestion of marine debris in other studies are usually sporadic (0–2 cases; Table S3). Only Blasi and Mattei (2017) reported significant mortality (38.6%) of loggerhead sea turtles associated to gastrointestinal occlusion due to the ingestion of anthropogenic debris and plastic bags. However, these authors did not provide data on the amount of debris ingested.

With regard to debris of anthropogenic origin, six studies have hitherto applied the MSFD protocol to analyse composition of ingestion by loggerhead sea turtles (Table 6). Unfortunately, data are not always presented in a comparable way. In particular, not all studies report 95% CI of FO%, the FO% of debris subcategories, the standard deviation of mean values, or even the size of the turtles (Table 6). Recently, Provencher et al. (2017) stressed the need for a proper implementation of guidelines to make full use of data across studies. Overall, the anthropogenic debris composition found in the present study is remarkably similar to that reported in previous surveys, with 'sheetlike' plastics and 'fragments' of hard plastic being the most common items, followed by 'threadlike' and 'foamed' plastics. Interestingly, plastic pellets of industrial origin are ingested infrequently (Table 6). The FO% of the categories 'rubbish' and 'pollutants' are much more variable among studies, perhaps reflecting a strong local component (Table 6).

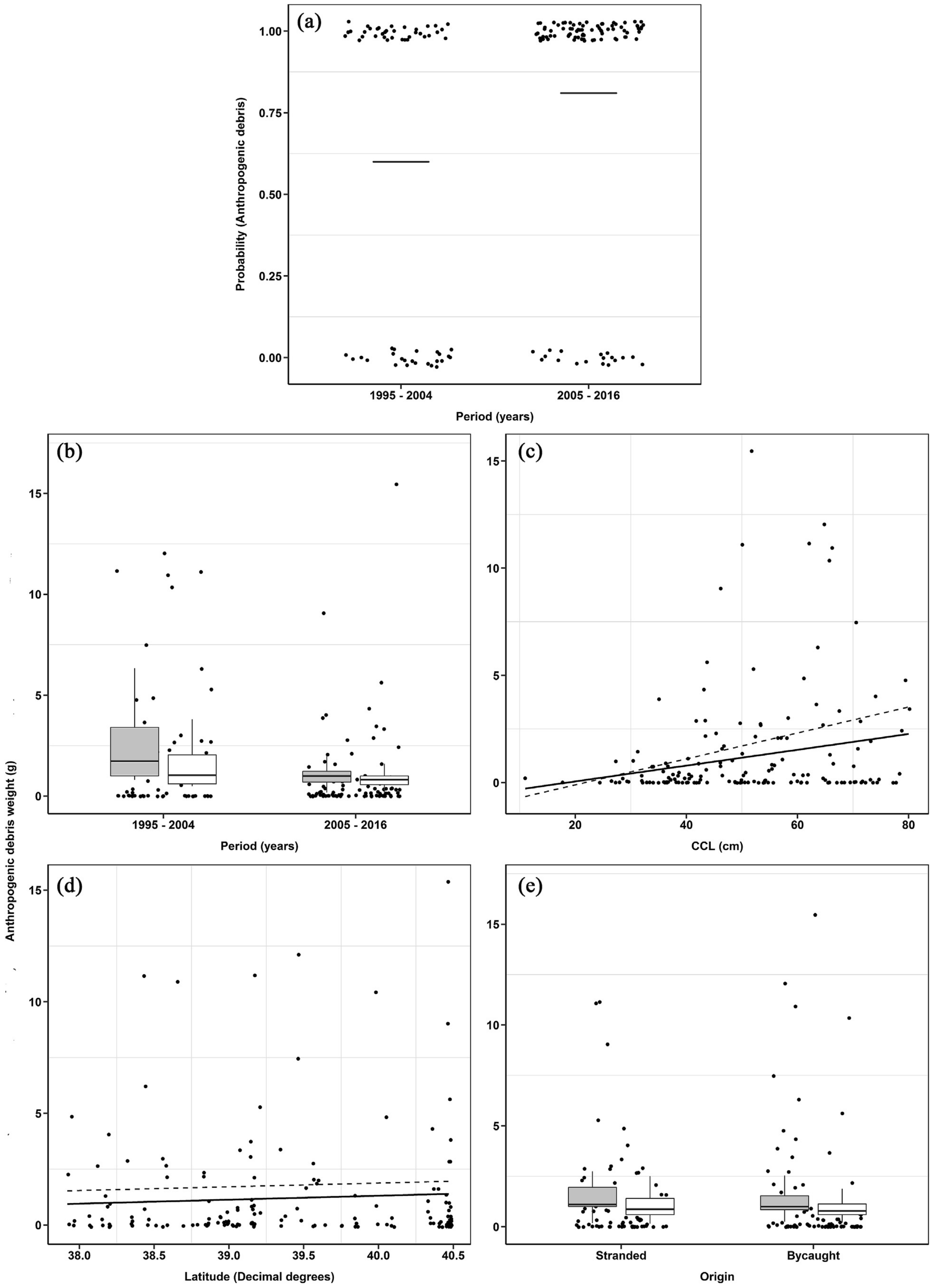

Fig. 3. Relation of the anthropogenic debris weight (in grams) in the digestive contents of 155 loggerhead sea turtles, *Caretta caretta* from Valencia region (East Spain) with the explanatory variables of the best-fitted ZAG model. (a) and (b) Period, (c) CCL, (d) Latitude and (e) Origin (stranding and bycaught turtles). Horizontal, solid lines in (a) show the probability of occurrence in both periods according to the expected values of the best-fitted ZAG model for the Bernoulli part. In (b), (c), (d) and (e), the dashed lines and grey boxes show the expected values of the best-fitted ZAG model only for the Gamma part, and the solid lines and white boxes show expected values of the best-fitted ZAG model (Bernoulli and Gamma parts together).

Table 5
Good Environmental Status (GES) scenarios proposed by Matiddi et al. (2017) applied to plastic ingestion in loggerhead sea turtles, *Caretta caretta*, from the Valencia region (East Spain). GES scenarios are calculated for early juveniles (CCL ≤ 40 cm) and for late juveniles (CCL > 40 cm) turtles. The methodology used to calculate GES scenarios can be found in the materials and methods section of the present paper. For comparison, total values of anthropogenic debris were included. The mean (±SD) was calculated including turtles without debris; whereas for the median and range only turtles with debris in their digestive contents were considered.

Ecological group	Sample size	Anthropogenic debris weight (g)				Plastic weight (g)				
		Frequency (%) (95% CI)	Mean (±SD)	Median	Range	Frequency (%) (95% CI)	Mean (±SD)	GES (%) (95% CI)	Median	Range
Early juveniles 1995–2016	41	82.9% (68.5–91.8)	0.349 (±0.669)	0.082	0.001–3.884	82.9% (68.5–91.8)	**0.345 (± 0.667)**	**26.8% (15.3–42.6)**	0.082	0.001–3.884
Early juveniles 1995–2005	3	–				–				
Early juveniles 2006–2016	38	84.2% (68.6–92.9)	0.353 (±0.689)	0.080	0.001–3.884	84.2% (68.6–92.9)	**0.348 (±0.687)**	**26.3% (14.0–42.0)**	0.080	0.001–3.884
Late juveniles 1995–2016	114	69.3% (60.1–77.3)	1.719 (±2.973)	0.337	0.001–15.463	64.9% (55.7–73.3)	**1.395 (±2.613)**	**29.8% (21.8–39.0)**	0.155	0.001–15.177
Late juveniles 1995–2005	60	60.0% (46.7–71.8)	2.059 (±3.255)	0.317	0.016–12.045	55.0% (42.2–67.6)	**1.468 (±2.664)**	**30.0% (19.6–42.9)**	0.115	0.016–11.145
Late juveniles 2006–2016	54	79.6% (66.8–88.6)	1.339 (±2.602)	0.340	0.001–15.463	75.9% (63.0–85.7)	**1.313 (±2.577)**	**27.8% (17.4–41.6)**	0.322	0.001–15.177

Table 6
Debris ingestion parameters in loggerhead sea turtles, *Caretta caretta*, from seven studies (including present study) that applied the protocol for sampling and quantification of marine debris proposed by Marine Strategy Framework Directive (MSFD). Debris categories (and their acronyms) are based on Van Franeker et al. (2011). Acronyms for MSFD sub-regions: Western Mediterranean (WM), Ionian Sea and the Central Mediterranean Sea (CM), Bay of Biscay and the Iberian Coast (IC) and Macaronesia (Mac). Other acronyms: Anthropogenic Debris Weight (ADW) and Anthropogenic Debris Items (ADI). Dash symbol indicates data not provided by corresponding authors.

Study	Present study	Matiddi et al. (2017)	Campani et al. (2013)	Camedda et al. (2014)	Casale et al. (2016)	Nicolau et al. (2016)	Pham et al. (2017)
Subregion **n**	WM (This study) 155	WM 120	WM 31	WM 121	CM 172	IC 95	Mac 24
Mean CCL (cm)	51.1 (14.9)	60.6	51.4 (12.2)	51.4 (12.4)	–	49.8 (9.3)	32.4 (20.2)
Mean ADW (±SD) (g)	1.32 (2.63)	1.30 (2.20)	2.01	0.23	0.48	1.35 (4.40)	1.07 (2.01)
Mean ADI (±SD)	10.6 (16.3)	16 (32.8)	16.5 (29.1)	2.8	10.6	9.7 (16.8)	15.8 (29.8)
Frequency (%) [95% CI]	71.0 [63.2–77.8]	85.0 –	71.0 –	14.0 –	100.0 –	59.0 –	83.0 –
Anthropogenic debris categories [frequency (mean weight in grams ± SD)]							
Industrial pellets	0	0	0	0.8 –	1.7 –	12.6 0.06 ± 0.23	– 0.00 ± 0.00
Sheet-like	60.6 0.35 ± 0.92	– 0.55 ± 1.1	– 0.69	12.4 –	82.0 –	45.3 0.29 ± 1.26	–
Thread-like	31.6 0.11 ± 0.44	– 0.15	– 0.02	4.1 –	25.6 –	24.2 0.13 ± 0.65	–
Foamed user	19.4 0.10 ± 0.41	– 0.05	– 0.01	1.7 –	2.3 –	5.3 0.00 ± 0.01	–
Fragments hard	38.7 0.39 ± 1.16	– 0.60 ± 1.1	– 1.06	9.1 –	58.7 –	4.2 0.00 ± 0.02	–
others	15.5 0.16 ± 0.54	– 0.10	– 0.13	0 –	13.4 –	3.2 0.01 ± 0.04	0 –
PLASTICS	69.0 1.11 ± 2.31	–	– 1.92	–	99.4 –	56.8 0.49 ± 1.50	–
Paper	2.6 0.00 ± 0.02	– 0.02	– 0.02	0	1.7 –	4.2 0.00 ± 0.01	–
Kitchen food	0	– 0.02	0	0	0 –	6.3 0.60 ± 3.38	–
Various	1.3 0.01 ± 0.04	– 0.05	– 0.09	0 0.00 ± 0.00	12.2 –	26.3 0.15 ± 0.46	–
Fish hooks	5.8 0.19 ± 1.19	0 –	– 0.04	0	0 –	1.1 0.03 ± 0.29	–
RUBBISH	9.7 0.20 ± 1.19	–	– 0.15	0	14.0 –	29.5 0.78 ± 3.51	–
coal/slag	0	– 0.02	0	1.7 –	0 –	3.2 0.01 ± 0.08	–
tar-lumps	5.8 0.01 ± 0.03	– 0.01	– 0.01	1.7 –	3.5 –	0	–
chemical	0	– 0.02	0	0	0 –	0	–
POLLUTANTS	5.8 0.01 ± 0.03	–	– 0.01	–	3.5 –	3,2 0.01 ± 0.08	–

4.2. Predictors of debris ingestion

According to ZAG models, 4 predictors have a substantial effect on the occurrence and/or mass of anthropogenic debris ingested by loggerhead sea turtles. ‘Period’ was selected in models for both occurrence and mass. However, its influence was opposite, i.e., debris occurred less frequently, but in larger amounts per turtle, in the period 1995–2004 compared with 2005–2016. While this pattern could reflect a temporal change in the amount and/or distribution of debris in the area, ‘period’ is also largely confounded by the putative cause of death and size of the turtles. In the first period, the main identified cause of stranding was bycatch associated to pelagic longline fishery, thus suggesting that these turtles were captured in oceanic waters (Tomás et al., 2008) and, surprisingly, they were significantly a little larger. On the contrary, reports of turtles accidentally caught by artisanal fisheries (trammel and bottom longline) and bottom trawling, which operate in neritic waters, have increased in the last decade along with a slight decrease in the size of the turtles. It seems clear, however, that unreported catches of turtles by artisanal fisheries and bottom trawling also occurred across the study period (Tomás et al., 2008; Domènech et al., 2015), perhaps accounting for a sizable portion of all stranded turtles found (Fig. 1). In summary, only the sample of turtles from the first period contained larger individuals captured in the oceanic realm. Therefore, we cannot rule out that the difference between periods can reflect, at least in part, corresponding differences in abundance and distribution of debris between oceanic vs. neritic areas. Likewise, we cannot rule out that the differences between periods are influenced in part by the size of the turtles. The model results indicate an influence of the size of the turtles in the amounts of debris in their gut contents, but we believe that in this case, the differences in the distribution of marine debris between oceanic and neritic realm may have a greater weight in the quantities of marine debris available for the turtles. For instance, in neritic areas, the amount of debris could be higher (especially in densely populated coastal areas, Suaria and Aliani, 2014), but more dispersed (Mansui et al., 2015) than in oceanic areas.

The weight of anthropogenic debris also increased significantly with CCL. The association between turtle size and amount of debris (natural, anthropogenic, or both) has been frequently examined in previous studies, with contrasting results. Most studies did not find any significant trend using, as dependent variable, presence/absence of anthropogenic debris (Casale et al., 2016); weight of anthropogenic debris (Lazar and Gračan, 2011; Schuyler et al., 2012; Camedda et al., 2014; Hoarau et al., 2014; Matiddi et al., 2017), volume of floating plastics (Tomás et al., 2002), or weight of anthropogenic plus natural debris (Nicolau et al., 2016). On the contrary, some authors found a significant positive correlation between CCL and weight of anthropogenic debris (Pham et al., 2017), weight of plastics (Campani et al., 2013), or volume of anthropogenic plus natural debris (Tomás et al., 2002). In addition, Plotkin and Amos (1990) and Schuyler et al. (2012) found a negative relationship between frequency of anthropogenic debris and CCL, and Clukey et al. (2017) found a negative relationship between body burden (g plastic/kg turtle) and straight carapace length.

If the turtles intake debris regularly, there will be different factors that could explain that larger turtles showed higher amounts of debris in the present study:

(1) The habitats used by turtles are traditionally linked to their life history. Due to their behaviour and distribution, turtles that inhabit oceanic areas increase the likelihood of ingesting floating marine debris (Schuyler et al., 2016). In general terms, the populations of loggerhead turtles that inhabit oceanic areas are the smallest (post-neonates and early juveniles). However, in several loggerhead sea turtle populations exists a dichotomy, with some turtles using oceanic areas in adult or sub-adult stages (Hatase et al., 2002; Hawkes et al., 2006), as is the case of the western and central Mediterranean Sea for late-juvenile loggerhead sea turtles (Tomás et al., 2001; Revellés et al., 2007; Casale et al., 2008).

(2) The preference for ingesting debris may change with turtle ontogeny. Loggerhead sea turtles feed opportunistically throughout their life cycle, taking advantage of any potential food in both oceanic and neritic areas (Tomás et al., 2001; Casale et al., 2008). This feeding strategy makes them vulnerable to the ingestion of marine debris, and the amounts of debris ingested can be consequence of the area they inhabit (Schuyler et al., 2012; Schuyler et al., 2016) rather than a preference related to turtle ontogeny.

(3) In the habitats where all turtle size classes meet, the amount of debris ingested would be explained by the intake ratio. This ratio increases with the size of the turtles. Although metabolic rate per kilogram of turtle decreases with the weight of the turtle, the absolute energy needs are greater as the turtle is larger (Wallace and Jones, 2008). Consequently, larger turtles consume higher amounts of food due to higher absolute energetic requirements hence increasing intake ratio.

(4) Furthermore, larger turtles have lower gape limitation and, therefore, they can swallow larger pieces of debris (Costa, 2009).

(5) Larger turtles retain food items for longer because they have longer intestines and need more time to complete digestion (Tomás et al., 2001; Costa, 2009). In fact, analysing data on transit of food items through loggerhead sea turtle guts reported in Di Bello et al. (2006), we found that the time of digestive food retention was significantly higher in turtles of greater weight ($r = 0.859$, $p = 0.006$, $n = 8$); although this tendency is not always observed (Valente et al., 2008).

According to these evidences, it is interesting to briefly discuss the reasons why other studies fail to find a positive relation between mass of debris ingested and size of the turtles. First, in some studies the range of sizes can be too narrow to detect it (Nicolau et al., 2016). Second, most of the studies are based on opportunistic turtle samples and use univariate tests (typically Spearman correlation) to explore the relationship between CCL and amount of debris, without considering the conjoint potential effect of other confounding variables. Third, if the density of debris in the environment is low, a ‘turtle size’ effect cannot easily be detected because the rate of debris consumption will also be low, and significant accumulation in larger turtles will hardly occur. Finally, there is the possibility that ontogenetic changes in habitat use and foraging behaviour can generate variability in the likelihood of contact with debris and/or the decision to consume them. However, the ontogenic dietary shift from oceanic to neritic areas described for the species in other areas seems to be less clear, or at least more flexible, in the western and central Mediterranean (Tomás et al., 2001; Casale et al., 2008).

We also detected a slight, but significant, latitudinal increase in the quantity of anthropogenic debris ingested in the study area. This pattern is probably related to a higher availability of anthropogenic debris at higher latitudes. Suaria and Aliani (2014) identified several major causes of entry of marine debris to the sea, including river outflows, size of coastal human populations, marine traffic and fishing grounds. Our findings are consistent with the operation of some of these factors. The mightiest river in Spain, the Ebro River, flows out into the sea at the north limit of the study area.

Also, coastal population size and marine traffic are larger in the north-half of the study area, mainly due to the location of the capital of the region, Valencia. However, most anthropogenic debris is persistent over very long periods, i.e., centuries, and can drift far from their origin (Ryan, 2014). Rather interestingly, oceanographic modelling also suggest that in the north-half of the study area there is a higher likelihood of debris retention and beaching due to ocean current dynamics (Mansui et al., 2015).

Finally, the results of the ZAG model based on AIC values showed that stranded turtles had a higher mass of anthropogenic debris compared with bycaught turtles. However, this effect was not statistically significant and, overall, we should interpret it with caution. Although we only included stranded turtles in low decomposition states (States 2–3, according to Vélez-Rubio and Tomas, 2016) which also were in good body condition, we cannot rule out that the sample contains some individuals affected by disease; these individuals would not necessarily reflect normal feeding behaviour (Casale et al., 2016).

4.3. Assessment of Good Environmental Status

Following the Marine Strategy Framework Directive (MFSD), Matiddi et al. (2017) proposed a methodology to obtain GES values from 'plastics' ingested by loggerhead sea turtles. For the period 2011–2014, Matiddi et al. (2017) estimated a GES value of 27% of turtles with more plastics than average (1.3 g). The GES value for late juvenile turtles obtained in our study for the period 2006–2016 is surprisingly similar, i.e., 27.8% of the turtles with more plastics than average (1.3 g). Our study also provides, for the first time, GES values for early-juvenile loggerhead sea turtles, with even lower figures (26.3% of turtles with more plastics than average, 0.35 g). Although based on a small sample size, 2005–2016 GES value obtained for early-juveniles can serve as the baseline to evaluate future trends in the level of plastics in the western Mediterranean.

The comparison between 1995-2005 and 2006–2016 GES values for late juveniles in our region revealed a slight decrease in the amount of plastics ingested by loggerhead sea turtles. Whether this positive result has resulted, at least in part, from the policies related to plastic usage recently applied (European Directive, 2008) is an open question. As noted above, the habitat where turtles were collected differed between the periods 1995–2005 and 2006–2016, with the former including many more individuals that presumably foraged in oceanic waters. The size of turtles was also smaller in the second period. Thus, the question arises as to whether GES values are strictly comparable between periods. We submit that, when possible, calculation of separate values of GES for neritic and oceanic turtles seems adequate given the differences in the feeding ecology of the loggerhead sea turtle (Bjorndal, 1997; Frick et al., 2009; Lazar et al., 2011). Matiddi et al. (2017) proposed separation of turtles according to CCL based on the assumption that early juveniles (CCL ≤ 40 cm) exploit different habitats than late juveniles (CCL > 40 cm). However, CCL appears to be a poor predictor of the feeding ecology of loggerhead sea turtles in the Western Mediterranean since turtles can be found in oceanic or neritic waters depending on food resources availability regardless of their size (Tomás et al., 2001; Casale et al., 2008). In our study, larger turtles were indeed captured in oceanic waters by pelagic-oceanic fisheries. Perhaps, the type of fishing gear of bycaught turtles and the epibiont fauna detected in turtles could be better indicators of exploitation of neritic or oceanic habitats (Casale et al., 2008, 2012).

Further studies are necessary to modify and improve the methodology proposed by Matiddi et al. (2017) and to evaluate the criteria used to assess the best GES indicator. For instance, the INDICIT consortium (https://indicit-europa.eu/) is currently developing a large network to collect standardized data to better define the indicator GES and criteria in order to support the European Commission for the implementation of the indicator "Litter ingested by sea turtles" in the OSPAR, the Barcelona RSCs and MSFD regions (Darmon et al., 2018).

5. Conclusions

Marine debris currently has a high occurrence in the digestive contents of juvenile loggerhead sea turtles that inhabit the western Mediterranean. However, the amounts ingested by this species are low and do not apparently pose a significant threat to the survival of their populations in the region. This species can be a good indicator of pollution in the Mediterranean Sea, as has been proposed by EU Marine Strategy Framework Directive through the standardized protocol for the collection of marine debris and the GES values proposed by Matiddi et al. (2017). For these purposes, it is necessary to take into account at least two considerations in future studies:

(1) To check the predictors that may be influencing the occurrence and amount of debris, mainly (a) the neritic or oceanic habitat of the turtles and (b) the biases associated with the use of stranded turtles (see Casale et al., 2016).
(2) To make a proper implementation of guidelines to make full use of data across studies and be able to do viable comparisons among them (see Provencher et al., 2017).

Acknowledgements

We thank the Wildlife Service of the Conselleria d'Agricultura, Medi Ambient, Canvi Climàtic i Desenvolupament Rural of the Generalitat Valenciana for logistic and financial support. We also thank the staff of the Marine Zoology Unit for technical assistance during the survey and two anonymous reviewers for their valuable comments on a previous version of this manuscript. JT and JAR are supported by EU projects INDICIT 11.0661/2016/748064/SUB/ENV.C2 and MEDSEALITTER (Interreg) CPI-18-014. FD is supported by a predoctoral grant UV-INV-PREDOC15-265927 of the Universitat de València.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2018.10.047.

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